Virological characterization and biological significance of the spike furin cleavage site in the Omicron variant of SARS-CoV-2 *in vitro* and *in vivo*

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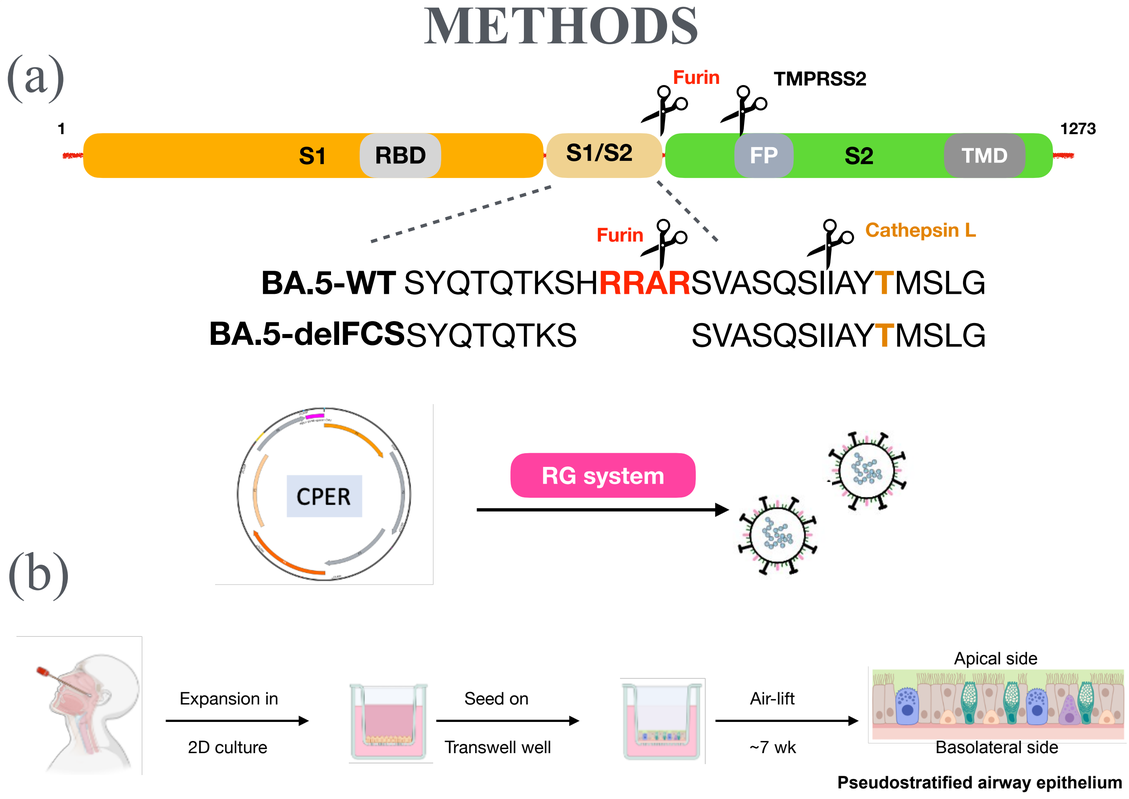
INTRODUCTION

Acquisition of a polybasic furin cleavage site (FCS) may provide SARS-CoV-2 with a unique ability to cross host barriers and facilitate human infections during the early stages of cross-species transmission. However, it is currently unclear whether the human adapted Omicron variant of SARS-CoV-2 requires FCS for efficient replication in mammalian cells.

METHODS



We used a reverse genetic system to generate a series of mutant viruses containing complete polybasic FCS deletion or various FCS mutations. We infected human airway epithelium (HAE) air-liquid interface cell-culture models (ALI) for single-cell temporal transcriptomic analysis. Additionally, a newly developed spatial transcriptomics technology was used to investigate tissue tropism of virus with variations in FCS and in situ virus-host interaction.

RESULTS

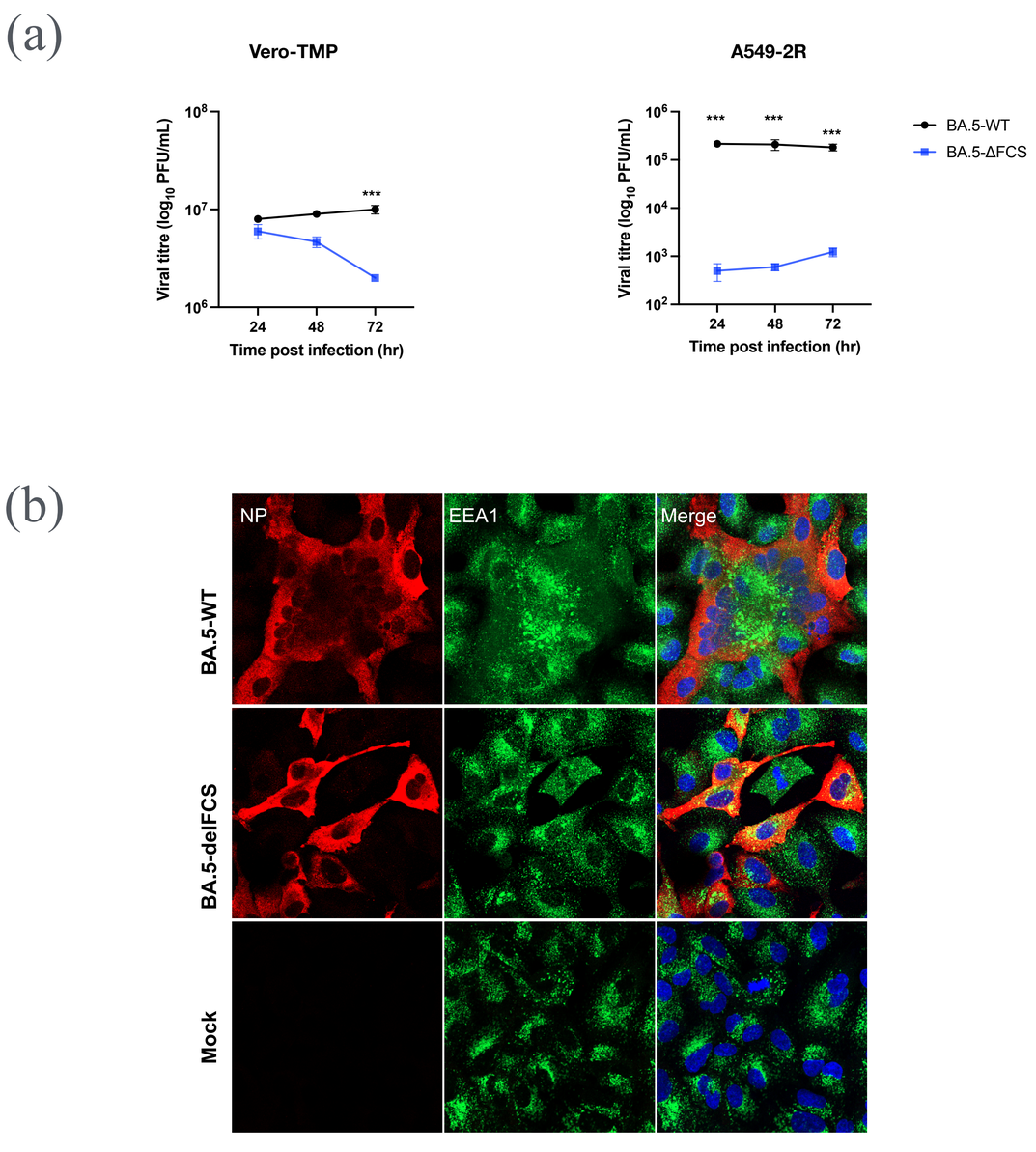


Fig 1. Comparison of BA.5-WT with del FCS viruses infection in cell lines.

(a) The two cell lines, Vero-TMP and A549-2R were infected with BA.5-WT or del FCS viruses at MOI=0.01. Virus without the FCS replicates to a lesser extent in the Vero-tmp cell, especially in later time points. In A549-2R cells, the replication efficiency of delFCS virus is significantly lower at all time points.

(b) Representative immunofluorescent images of A549-2R cells infected with BA.5-WT and del FCS at 24 hpi taken at high magnification power (40x). Infected cells were identified using a primary antibody against viral NP (red). Primary antibodies against EEA1(green) were used. The FCS deletion virus does not lead to cell-cell fusion as the wild-type virus does.

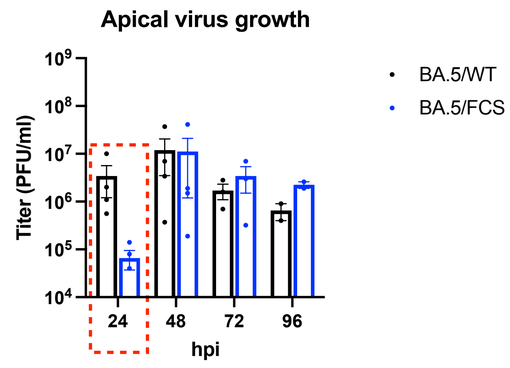


Fig 2. BA.5-WT replicated more efficiently than BA.5- del FCS virus in reconstituted human nasal epithelium.

To model the actual upper respiratory compartment of the human airways, we develop the 2D nasal organoid system. Briefly, we cultured nasal swabs on transwell membranes and induced differentiation using a well-established protocol. After around 2 months of differentiation, we obtained 2D organoids which can be infected.

We assess the replication efficiency of wild-type and FCS deletion virus using this system. First of all, we observe that SARS-CoV-2 replicates very efficiently in nasal cells. Secondly, the FCS deletion virus also shows less efficient replication in the first 24 hours. Surprisingly, the deletion virus catches up in replication in later time points, suggesting that the absence of FCS only affects the entrance of the virus but not replication once the virus is in the cells.

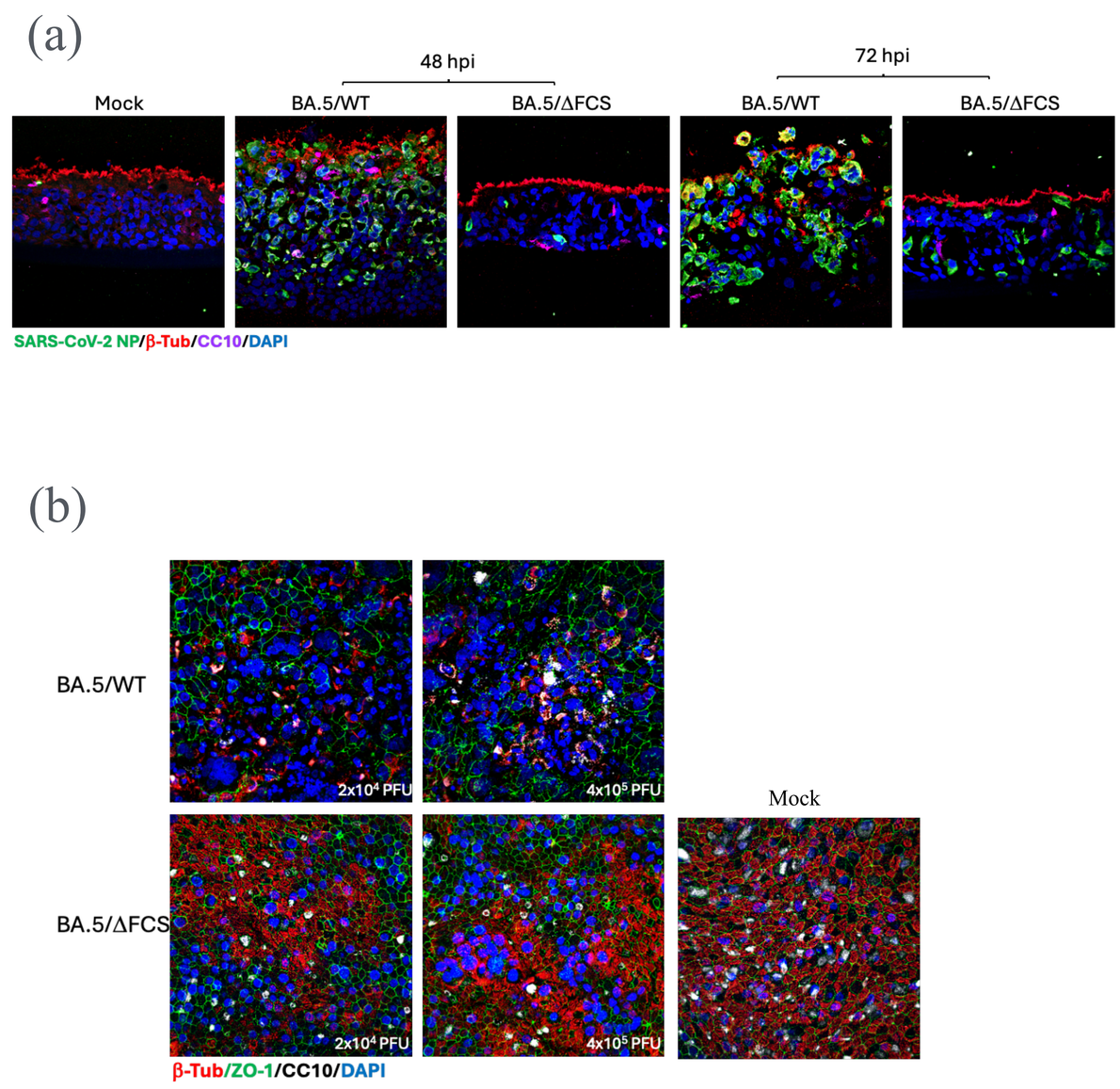


Fig 3. Cellular tropism of SARS-CoV-2 virus.

Representative immunofluorescent images of infected nasal ALI cultures with BA.5-WT and del FCS at 24 hpi taken at high magnification power (40x).(a) Infected cells were identified using a primary antibody against viral NP (green). Primary antibodies against β-Tubulin (red), and CC10 (purple) were used to identify ciliated cells and club cells coorespondingly. It is shown that ciliated cells are significantly infected whereas Club cells are not infected. Moreover, the WT virus infects the epithelium to a faster and greater extent than FCS deletion virus at 48 hpi. At 72hpi, the epithelium suffers significant cell death from wild-type virus infection. It is observed that a substantial loss of ciliated cells in wild-type virus infection. On the other hand, the epithelium remains largely intact from infection with FCS deletion virus. (b) Primary antibodies against β-Tubulin (red), and CC10 (grey) were used to identify ciliated cells and club cells coorespondingly. The epithelium integrity was shown by staining ZO-1, which is a junction protein between cells. As seen, wild-type virus infection leads to significant cell-cell fusion that is reflected by the irregular ZO-1 staining.

CONCLUSIONS

Our study demonstrated that HAE ALI is a suitable model for studying molecular basis of host adaption by SARS-CoV-2 coronavirus to address the importance of polybasic FCS during infection. The FCS is required for cross-species transmission of SARS-CoV-2 virus at the early stage of human infections and is stably retained for efficient replication in the most recent Omicron variant.

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